Interleukin-1 Receptor Antagonist Allele 2 and Familial Alopecia Areata

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Alopecia areata affects 1%–2% of the population and is hypothesized to be an autoimmune, organ specific T-cell mediated reaction directed against the human hair follicle. It is characterized by loss of hair in patches (alopecia areata) with progression in some individuals to total loss of scalp hair (alopecia totalis) or to loss of all scalp and body hair (alopecia universalis). The interleukin-1 receptor antagonist (IL-1RN) gene was found to be associated with more severe clinical outcome in several chronic inflammatory diseases, including alopecia areata. The IL-1RN*2 allele was found to be associated with alopecia areata severity in a British case-control study. In this paper, we analyzed alopecia areata probands in a family-based sample (n = 131 parent–offspring trios) to study the association between alleles of the IL-1RN and various phenotypes of alopecia areata. In considering all patients with any form of alopecia areata, no association was found with IL-1RN. IL-1RN*2 allele was not associated with alopecia totalis and alopecia universalis. A borderline association was observed between IL-1RN and patchy alopecia areata but it was not statistically significant ($p = 0.06$). We also observed an association between IL1-RN*1 allele and patchy alopecia areata ($p = 0.045$). *Key words: association tests/cytokines/genotypes/hair follicles. J Invest Dermatol 118:335–337, 2002*

Alopecia areata (AA) is hypothesized to be an autoimmune, organ specific T-cell mediated reaction directed against the human hair follicle. It is characterized by loss of hair in patches (AA), total loss of scalp hair (alopecia totalis, AT), or total loss of scalp and body hair (alopecia universalis, AU). AA occurs in 1%–2% of the population, and has a strong hereditary component with family history ranging from 10% to 25% in different studies (Gollnick and Orfanos, 1990). Significant associations in HLA class II alleles (DR*5 and DQ*03) have been reported in case-control (Duvic *et al*, 1995) and family-based studies (de Andrade *et al*, 1999; Duvic *et al*, 2001). Twin studies have shown a concordance rate of 55% AA in identical twins but not in fraternal twins (Jackow *et al*, 1998). AA is widely regarded as an autoimmune disease where the anagen hair follicles apparently become the target for an immune cell attack.

The local production of pro-inflammatory cytokine interleukin 1 (IL-1) and its receptor antagonist (IL-1RA) is one of the major determinants of an inflammatory response in the skin. Interleukin-1α (IL-1α) found in high levels in keratinocytes can be released following trauma, ultraviolet B irradiation, and local infection. It has been previously shown that decreased levels of IL-1RA are present in other inflammatory skin lesions including psoriatic plaques (Kristensen *et al*, 1992). Therefore, IL-1 may play a role in AA through a direct growth-inhibitory effect on hair follicles. The gene for IL-1RA, also called IL-1RN, is located on the long arm of chromosome 2 (2q14.2). The variable number of tandem repeats (VNTR) in intron 2 of the gene IL-1RN has five alleles, comprising 2–6 repeats of an 86 bp sequence (Tarlow *et al*, 1994). The IL-1RN*2 allele has been found to be associated with more severe clinical outcome in several chronic inflammatory diseases, including systemic lupus erythematosus (Blakemore *et al*, 1994), ulcerative colitis (Mansfield *et al*, 1994), and AA (Tarlow *et al*, 1994; Cork *et al*, 1995).

The primary goal of this study was to evaluate whether specific IL1-RN alleles are associated with AA in a family-based sample, as previously published for sporadic cases.

MATERIALS AND METHODS

Data collection Cases were identified from several dermatology clinics at Texas Medical Center, Houston, TX, and through the web-site http://www.mdanderson.org/departments/alopecia. DNA was extracted from peripheral blood using standard protocols according to the manufacturer's instructions (Stratagene, La Jolla, CA). A total of 143 families with 246 unaffected and 240 affected individuals were genotyped for the IL-1RN gene (Duvic *et al*, 2001).

Polymerase chain reaction (PCR) analysis We have amplified a region from human genomic DNA in the IL-1RN gene using PCR primers as described in Tarlow *et al* (1994): 5′-TCCTGGTCT-GCAGGTAA-3′ and 5′-CTCAGCAACACTCCTAT-3′. PCR conditions were as follows: a denaturing step at 94°C for 3 min, 40 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The final PCR products were analyzed on a 2.5% agarose gel and sized by comparison to ØX174 digested with Hinf-1 (Promega, Madison, WI) and 100 bp DNA step ladder (New England BioLabs, Beverly, MA) under ethidium bromide visualization.

Statistical analysis The occurrence of each allele occurring in the family-based sample was expressed as a percentage of the total number of alleles to give an allele frequency. The frequencies were estimated from the pedigree data using the method proposed by Boehnke (1991) with

Manuscript received July 16, 2001; revised October 23, 2001; accepted for publication November 5, 2001.

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Table I. Distribution of the IL-1RN allele frequencies from affected family members including the probands

	Frequency (%)			
Alleles	AA affected (n = 240)	Patchy AA (n = 125)	AT (n = 27)	AU (n = 88)
1	72.92	75.20	72.22	69.89
2	23.75	21.20	24.07	27.27
3	2.08	2.40	1.85	1.70
4	1.25	1.20	1.85	1.14

Table II. Gene carriage rate of IL-1RN alleles among affected and unaffected family members

	Frequency (%)			
Alleles	Unaffected (n = 246)	Patchy AA (n = 125)	AT (n = 27)	AU (n = 88)
1	91.46	92.00	96.63	92.05
2[a]	44.08	36.80	44.44	48.86
3	3.25	4.80	3.70	3.41
4	1.63	2.40	3.70	2.27

[a]Published frequencies for gene carriage rate for allele 2: unaffected, 41%; patchy AA, 44%; AT, 66%; AU, 77%. Gene carriage rate for alleles 1, 3, and 4 were not presented (Tarlow *et al*, 1994).

the ILINK program of the LINKAGE package (Lathrop *et al*, 1984). The rate of gene carriage is defined as the number of individuals carrying at least one copy of a specific allele as a proportion of the total number of individuals and was also calculated. χ^2 tests were performed on the frequencies when appropriate. Family-based association tests between AA cases and their parents were performed. From the 143 families, only 131 trios (one or both parents and one affected offspring) were eligible for the analysis (parent and child sharing different heterozygous genotype).

The transmission disequilibrium test (TDT) was performed to evaluate whether the IL-1RN gene is associated with AA (Spielman *et al*, 1993). First, the χ^2 marginal homogeneity test was applied to evaluate whether there is an association between IL-1RN and AA considering multiple alleles at this gene. Second, the Ewen's χ^2 test was applied to evaluate specific association from each IL-1RN allele and AA.

RESULTS

The allelic distributions for all affected probands (n = 240) and for the phenotypic severity types of AA, AT, and AU are broken down in **Table I**. Although there are five different alleles of IL-1RN (1, 410 bp; 2, 240 bp; 3, 500 bp; 4, 325 bp; 5, 595 bp), allele 5 was not present in these samples. The allele frequencies calculated using either the entire family-based sample or the subset of the unaffected members were not different from each other or from Caucasian controls published in the literature ($p > 0.10$) (Tarlow *et al*, 1994).

The frequency of allele 2 was 23.75% in the AA individuals ($p = 0.65$) in comparison to a frequency of 25.20% in unaffected family members. In 125 individuals characterized as having patchy AA, the frequency of allele 2 was 21.20% compared to 24.07% and 27.27% in patients characterized as having AT or AU, respectively (**Table I**). No statistically significant difference was found between these three groups ($p = 0.15$). Interestingly the frequency of allele 2 in all patients with any form of AA in our sample (23.75%) was much lower than the frequency reported elsewhere (32.2%, Tarlow *et al*, 1994). We also observed that this difference is statistically significant ($p = 0.03$).

The rate of gene carriage of the various IL-1RN alleles is shown in **Table II**. The gene carriage of allele 2 was 36.8% for patchy AA, 44.44% for AT, and 48.86% for AU patients. Thus, there was an increased gene carriage frequency of allele 2 in the more severe forms of AA. These values were not as high as those published by Tarlow *et al* of 44%, 66%, and 77%, respectively (**Table II**). The observed allele 2 gene carriage rate in unaffected related individuals (44.08%) was slightly higher than Tarlow's controls (41%). In particular, the allele 2 gene carriage rate in all AA cases (36.8%) was much lower than observed by Tarlow *et al* but it was not significant ($p > 0.10$).

The association test (TDT) was performed using parent–offspring trios in which the control for the case was the unaffected parent. No association was found between the IL-1RN gene and AA as a trait in all affected individuals ($p = 0.29$). Nor was an association found between any of the four alleles of the IL-1RN gene and AA. Specifically, the p-value for allele 2 was 0.33. Subsequently, TDT analyses were performed for all AA patients as well as various AA subsets – patchy AA and severe AA (AT and AU) – for each allele and for all alleles combined. No statistically significant associations were found for patchy AA ($p = 0.06$), AT ($p = 0.67$), and AU ($p = 0.88$). The IL-1RN allele 2 was found not to be associated with patchy AA ($p = 0.07$), with AT ($p = 0.65$), or with AU ($p = 1.00$). We did observe a borderline statistically significant association, however, between IL-1RN allele 1 and patchy AA ($p = 0.045$).

DISCUSSION

The genetic mechanisms of AA seem to be polygenic, where several genes play a role in determining the disease susceptibility. Genetic factors probably interact with environment factors, such as infection, to trigger the onset of disease, whereas other factors may determine how long the disease persists and how severe it becomes. As in other autoimmune diseases, the HLA locus is a major disease determinant (Spielman *et al*, 1993; Duvic *et al*, 1995, 2001; de Andrade *et al*, 1999).

The severity of an inflammatory response to an environmental trigger may also be determined by the balance of pro-inflammatory and anti-inflammatory cytokines. IL-1α is a major pro-inflammatory cytokine in the epidermis. The potent pro-inflammatory properties of IL-1α in the skin are balanced by the intracellular IL-1RA, which is also present in large quantities in keratinocytes. It seems likely that a relative deficiency of the intracellular IL-1RA could lead to the development of chronic inflammatory skin diseases.

Our study confirmed the findings by Blakemore *et al* (1996) that the four-repeat (IL1-RN*1) and two-repeat (IL-1RN*2) alleles are the most commonly found alleles in human subjects. The other alleles also occur at a combined frequency of less than 5% (Blakemore *et al*, 1996). Despite the previous report of a strong association between severity of AA and IL-1RN allele 2 (Tarlow *et al*, 1994; Cork *et al*, 1995), our study failed to show the same association among AA patients many of whom had a positive family history of AA. Of note, the relative frequencies of each IL-1RN allele were similar between the published control subjects, our controls taken from unaffected family members, and the group of affected plus unaffected individuals. Whereas Tarlow observed a 32.2% allelic frequency of allele 2, we found that only 23.75% of all our AA subjects carried this allele ($p = 0.03$). This was the major difference between the two groups of AA subjects.

We did not observe a significant increase in the frequency of allele 2 in our 240 AA subjects. Based on the former report by Tarlow *et al* we would have expected an even greater increase in allele 2 as some of the subjects in this study also were related to each other. On the other hand, we did observe a new association between IL-1RN allele 1 and patchy AA that was of borderline statistical significance.

There are several possibilities to explain the difference between the results observed by Tarlow *et al* (1994) and this new cohort of familial AA patients. First, the study designs were not equal. Tarlow *et al* used a case-control design and ours is a family-based design in

which cases were compared with their unaffected parents. Second, the source of the patients was different (i.e. U.K. *versus* U.S.A.). Nonetheless, our unaffected control relatives had the exact same allelic frequencies as did Tarlow's unrelated control subjects and the numbers of each were similar. Finally, the sample size of AA cases in Tarlow's paper ($n = 90$) was smaller than ours ($n = 240$). For example, he included only 18 AU patients compared to the 88 patients with AU in this study. The confidence interval for his odds ratio was 1.6–15.7, which is wide probably due to the small sample size. If anything, our subjects should have amplified the allelic frequency results because they were related to the controls in family studies. Thus, we conclude that the observed difference between the results of Tarlow *et al* and our results is due to the presence of different genes underlying the susceptibility to sporadic and familial AA.

This work was partially supported by NIAMS grants R03-AR45789 and N01-AR02249, and ongoing research support from The National Alopecia Areata Foundation.

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